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Protein synthesis in rabbit reticulocytes: Characteristics of a postribosomal supernatant factor that reverses inhibition of protein synthesis in heme-deficient lysates and inhibition of ternary complex (Met-tRNA$_f^{Met}$·eIF-2·GTP) formation by heme-regulated inhibitor*

(translational regulation/Met-tRNA$_f^{Met}$ binding factor/eukaryotic initiation factors)

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ABSTRACT **During heme deficiency in reticulocyte lysates, a translational inhibitor (heme-regulated inhibitor, HRI) that blocks polypeptide chain initiation is activated. HRI is a protein kinase that specifically phosphorylates the 38,000-dalton subunit of the Met-tRNA$_f^{Met}$ binding factor, eIF-2. Phosphorylation of eIF-2 by HRI prevents its interaction with at least two additional factors, resulting in a net reduction in formation of ternary complex (Met-tRNA$_f^{Met}$·eIF-2·GTP) and AUG-dependent transfer of Met-tRNA$_f^{Met}$ to 40S ribosomal subunits. A factor (sRF) that reverses protein synthesis inhibition in heme-deficient lysates has been purified from reticulocyte postribosomal supernatant. sRF also reverses the inhibition of ternary complex formation by HRI in a fractionated system. The ternary complex inhibition reversal activity and the protein synthesis inhibition reversal activity cosediment at 12.5 S upon glycerol density gradient centrifugation, and both activities are sensitive to heat or *N*-ethylmaleimide. Purified sRF does not dephosphorylate eIF-2 whose phosphorylation has been catalyzed by HRI, nor does the sRF prevent the phosphorylation of eIF-2 by HRI in a fractionated system. sRF stimulates ternary complex formation by both phosphorylated and nonphosphorylated eIF-2. These observations suggest that the sensitivity of protein synthesis to phosphorylation of eIF-2 by HRI may be modulated by the concentration and activity of sRF.**

During heme deficiency in reticulocyte lysates, protein synthesis shuts off abruptly due to activation of a latent protein synthesis inhibitor, the heme-regulated inhibitor (HRI) (9–19). HRI is a protein kinase that specifically phosphorylates the 38,000-dalton subunit of the Met-tRNA$_f^{Met}$ (tRNA$_f^{Met}$ will be abbreviated to tRNA$_f$) binding factor, eukaryotic initiation factor 2 (eIF-2), producing eIF-2(P) (20–24). Phosphorylation of eIF-2 does not inhibit its activity *per se* but prevents its interaction with at least two factors: Co-eIF-2B (or ternary complex dissociation factor, TDF) (25, 26) and Co-eIF-2C (1, 7). Co-eIF-2B is required for AUG-dependent Met-tRNA$_f$ binding to 40S ribosomal subunits (4). Co-eIF-2C relieves the Mg^{2+} inhibition of ternary complex formation, resulting in an apparent stimulation (1, 7). Factors similar to Co-eIF-2C have been reported by deHaro *et al.* (27, 28) and Ranu and London (29). Phosphorylation of eIF-2 by HRI thus results in a net reduction of ternary complex formation and loss of efficient Met-tRNA$_f$ transfer to 40S ribosomal subunits.

The inhibition of protein synthesis in heme-deficient lysates can be overcome by the addition of the ribosomal salt (0.5 M KCl) wash or a partially purified preparation of eIF-2 (30–33). We have reported (8) that this activity (protein synthesis inhibition reversal factor from ribosomes, rRF) is not due to eIF-2 nor is it correlated with eIF-2 activity or concentration. rRF was resolved from the bulk of eIF-2. Also, homogeneous eIF-2 was inactive in reversal of protein synthesis inhibition in heme-deficient lysates.

Another factor that reverses protein synthesis inhibition in heme-deficient lysates has been isolated from reticulocyte postribosomal supernatant (34–36). In this paper, we describe further purification and characterization of this factor, protein synthesis inhibition reversal factor from supernatant (sRF).

sRF efficiently restores the protein synthesis activity of heme-deficient lysates to the level observed in the presence of hemin and also reverses the inhibition of ternary complex formation by HRI in a fractionated system. The sRF activities for ternary complex inhibition reversal and for protein synthesis inhibition reversal cosediment at 12.5 S upon glycerol density gradient centrifugation, and both activities are sensitive to *N*-ethylmaleimide and heat. sRF neither dephosphorylates eIF-2(P) nor blocks phosphorylation of eIF-2 by HRI in a fractionated system. sRF stimulates ternary complex formation by both phosphorylated and nonphosphorylated eIF-2.

MATERIALS AND METHODS

Materials. The materials were obtained from the following sources: [^{14}C]leucine [289–355 mCi/mmol (1 Ci = 3.7 × 10^{10} becquerels)] from New England Nuclear; [^{35}S]methionine (900 Ci/mmol) from Amersham; [γ-^{32}P]ATP (2.7–3.1 Ci/mmol) from ICN; PM-10 ultrafiltration membranes from Amicon, Lexington, MA; GTP-agarose from Sigma. The sources of most of the other materials used in these studies have been described (8).

Preparations of Rabbit Reticulocyte Lysates and Assay of Protein Synthesis. The procedures for the preparation of reticulocytes and reticulocyte lysates and the incubation mixtures for protein synthesis have been described (14, 20). Protein synthesis was assayed by the incorporation of [^{14}C]leucine into hot trichloroacetic acid-insoluble protein. The specific activity

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Abbreviations: The present nomenclature for the peptide chain eukaryotic initiation factors (eIFs) (1), their past nomenclature (shown in parentheses) (2) as used by our laboratory, and their characteristic functions are: eIF-2 (EIF-1), Met-tRNA$_f^{Met}$ binding factor (3, 4); eIF-2(P), phosphorylated eIF-2 (phosphorylation catalyzed by heme-regulated translational inhibitor); Co-eIF-2A (Co-EIF-1), stimulates Met-tRNA$_f^{Met}$ binding to eIF-2 (5, 6); Co-eIF-2B (EIF-2, TDF), promotes dissociation of ternary complex at high Mg^{2+} (4); Co-eIF-2C (EIF-2B), reverses Mg^{2+} inhibition of ternary complex formation (7); HRI, heme-regulated translational inhibitor; rRF, ribosomal salt wash factor that reverses protein synthesis inhibition in heme-deficient lysates (8); sRF, postribosomal supernatant factor that reverses protein synthesis inhibition in heme-deficient lysates. tRNA$_f$, tRNA$_f^{Met}$; NaDodSO$_4$, sodium dodecyl sulfate; MalNEt, *N*-ethylmaleimide.

* This paper is no. 25 in a series. Paper no. 24 is ref. 1.

of the [^{14}C]leucine used is shown in the figure legends. The KCl concentration in the lysates was 80 mM in all cases. Incubation was at 30°C for 30 min unless otherwise indicated. Three different reaction volumes were used (12, 25, and 50 μl) for convenience in particular experiments. Specific activities of [^{14}C]leucine and aliquot size were adjusted so that the amounts of [^{14}C]leucine incorporated in the aliquots were comparable in all three systems.

Sodium Dodecyl Sulfate ($NaDodSO_4$) Gel Electrophoresis and Autoradiography. Electrophoresis of proteins in $NaDodSO_4$ polyacrylamide gels was performed according to the method of Laemmli (37). The gels contained 10% acrylamide (10% acrylamide, 0.27% *N,N'*-methylenebisacrylamide). The separating gels were formed as 1.5 × 100 × 150-mm slabs, with 1.5-cm stacking gels (5% acrylamide). Electrophoresis was performed at 150 V for 3½ hr in a Bio-Rad model 220 electrophoresis apparatus. The gels were stained with Coomassie brilliant blue. Autoradiography was performed as described with Kodak X-Omat R film (21).

Protein Determination. Protein concentration was determined according to the method of Bradford (38) as modified by Spector (39) with bovine serum albumin as the standard.

Preparation of sRF. The sRF was prepared as described by Gross (35), except that 10 mM Tris·HCl, pH 7.8 was used instead of Hepes, and the DEAE-cellulose column was eluted by using 0.1 M, 0.2 M, 0.3 M, and 0.5 M KCl. The bulk of the sRF activity eluted with the 0.2 M KCl wash. The active fractions were pooled and concentrated by ultrafiltration. The concentrated sRF (1.5 ml, 14 mg/ml) was loaded on a 1.2 × 4.8-cm GTP-agarose column equilibrated with buffer A (10 mM Tris·HCl, pH 7.8/1 mM dithiothreitol/0.1 mM EDTA/0.1 M KCl). The bulk of the sRF activity does not bind to the column and elutes in the void volume. The active fractions were pooled and concentrated by dialysis against 80–90% saturated ammonium sulfate as described by Schreier *et al.* (40). The concentrated sRF was dissolved in buffer A containing 10% (vol/vol) glycerol at a protein concentration of 4–8 mg/ml and stored at −96°C.

RESULTS

The sRF was purified by using DEAE-cellulose and GTP-agarose column chromatography. The bulk of sRF activity was not retained on the GTP-agarose column, whereas almost all of eIF-2 activity was bound and was later eluted by using 0.1 M GTP (Fig. 1). The GTP-agarose-purified sRF preparation was stored at −96°C. Under this condition, the preparation remained active for at least a month.

GTP-agarose-purified sRF efficiently reversed protein synthesis inhibition in heme-deficient lysates (Fig. 2). The amount of sRF giving maximum reversal (86 μg) (Fig. 2) bound only 0.31 pmol of [^{35}S]Met-tRNA$_f$ in the presence of GTP in the Millipore filtration assay. $NaDodSO_4$ gel electrophoresis result (data not shown) also confirmed the removal of nearly all eIF-2 from sRF by GTP-agarose chromatography.

Phosphorylation of eIF-2 by HRI inhibits its interaction with Co-eIF-2C, resulting in a net reduction in ternary complex formation when assayed in the presence of Mg^{2+} (1, 7). We investigated the effect of sRF on inhibition of ternary complex formation by HRI. In these experiments we used partially purified eIF-2 preparations (eIF) [fraction II (41)] that contained Co-eIF-2C activity.

As shown in Table 1, phosphorylation of eIF-2 by HRI inhibited its ability to form the ternary complex. sRF stimulated ternary complex formation by both phosphorylated and nonphosphorylated eIF-2. This stimulation of eIF-2 activity by sRF was observed even if sRF was added after eIF-2 had been phosphorylated by HRI (Table 1, experiment 2).

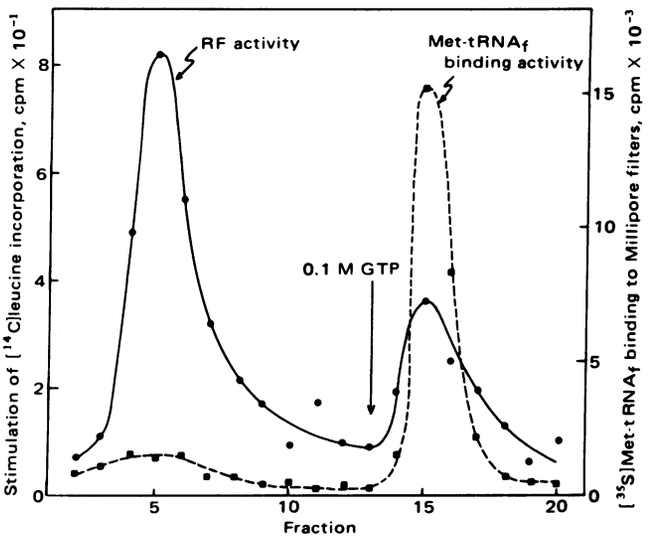

FIG. 1. GTP-agarose chromatography of sRF. [^{35}S]Met-tRNA$_f$ binding was determined with 2-μl aliquots from fractions. RF activity was determined with 2-μl aliquots added to 10-μl protein synthesis reaction mixtures. [^{14}C]Leucine incorporation was determined in 10-μl aliquots. The specific activity of the [^{14}C]leucine was 125 cpm/pmol. This lysate incorporated 7000 cpm of [^{14}C]leucine in the absence of hemin and 30,000 cpm in the presence of 40 μM hemin. The protein concentration of the peak fraction of sRF was 1 mg/ml.

In order to determine whether ternary complex inhibition and protein synthesis inhibition reversal activities were due to the same factor, sRF was analyzed by glycerol density gradient centrifugation. Both activities sedimented (Fig. 3) at 12.5 S, corresponding to an approximate molecular weight of 370,000. The ternary complex inhibition reversal activity, shown here as stimulation of ternary complex formation in the presence of

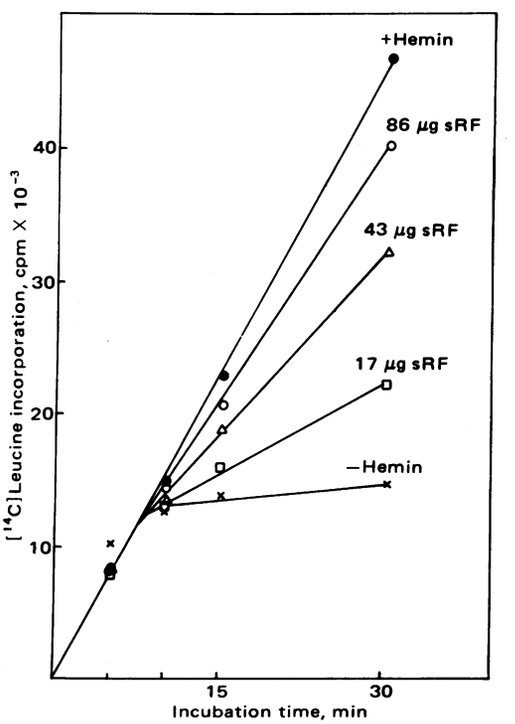

FIG. 2. Kinetics of protein synthesis in the presence of sRF. Reversal of protein synthesis inhibition in heme-deficient lysate by sRF. Protein synthesis was assayed in 50-μl reaction mixtures. The specific activity of the [^{14}C]leucine was 170 cpm/pmol. [^{14}C]Leucine incorporation was determined in 10-μl aliquots. Where indicated, the concentration of hemin used was 40 μM.

Table 1. Reversal of HRI inhibition of ternary complex formation by sRF

Additions	ATP	[^{35}S]Met-tRNA$_f$ bound, pmol −HRI	+HRI
	Experiment 1		
eIF	−	2.11	2.30
sRF	−	0.51	0.49
eIF + sRF	−	3.30	3.42
eIF	+	2.39	1.00
sRF	+	0.39	0.44
eIF + sRF	+	3.08	2.95
	Experiment 2		
eIF	+	1.75	0.94
eIF + sRF (stage I)	+	2.35	2.11
eIF + sRF (stage II)	+	—	2.25

Standard Millipore filtration assay conditions were used. The incubation mixtures in Exp. 1 contained, in a total volume of 50 μl: 30 mM Tris·HCl at pH 7.8; 130 mM KCl; 15 μg of bovine serum albumin; 3 mM dithiothreitol; 1.5 mM magnesium acetate; and, where indicated, 0.2 mM ATP, 15 μg of eIF [fraction II (41)], 0.8 μg of HRI, and 16 μg of sRF. The reaction mixtures were incubated at 37°C for 10 min (stage I). Ten microliters of 2 mM GTP (final concentration 0.27 mM) and 8–10 pmol of [^{35}S]Met-tRNA$_f$ (15 μl) were added and the incubation was continued at 37°C for 5 min (stage II). The reaction mixtures were assayed by Millipore filtration as described (41). The sRF preparation (16 μg) used in this experiment bound 0.3 pmol of [^{35}S]Met-tRNA$_f$ in the absence of GTP. The reaction conditions in Exp. 2 were the same as above except that sRF (16 μg) was added at either stage I or stage II.

HRI and ATP, correlated well with RF activity. The downward trend of the RF activity profile was due to inhibition of protein synthesis by increasing concentrations of glycerol. In this case and as reported previously (8), amino acid incorporation decreased linearly with glycerol concentration when blank gradients were used. Addition of 5 μl of solution containing 30% glycerol (concentration at the bottom of the gradient) lowered [^{14}C]leucine incorporation to approximately 60%. The amino acid incorporation activity of gradient fractions 1–6 (Fig. 3) was the same as the control within experimental error.

In order to investigate whether sRF contained phosphatase activity, the effect of sRF on phosphorylation of eIF-2 by HRI was analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis and autoradiography. The results are shown in Fig. 4. Comparison of the kinetics of phosphorylation of eIF-2 in the absence (tracks 6–8) and presence (tracks 9–11) of sRF clearly shows that sRF did not reduce the amount of phosphorylation

Table 2. Effects of addition of sRF and Co-eIF-2C on ternary complex formation by eIF-2(P)

Addition	[^{35}S]Met-tRNA$_f$ bound, pmol
None	0.15
sRF (8 μg)	0.58
sRF (12 μg)	0.69
Co-eIF-2C (2 μg)	0.12
CoeIF-2C (5 μg)	0.21

Standard Millipore filtration assay conditions were used. The incubation mixtures contained, in a total volume of 75 μl: 20 mM Tris·HCl at pH 7.8; 87 mM KCl; 15 μg of bovine serum albumin; 2 mM dithiothreitol; 1 mM magnesium acetate; 0.27 mM GTP; 8–10 pmol of [^{35}S]Met-tRNA$_f$; and eIF-2(P) [prepared as described by Das *et al.* (1)]. Reaction mixtures were incubated at 37°C for 5 min, then assayed by Millipore filtration as described (1). The eIF-2(P) preparation used bound 0.88 pmol of [^{35}S]Met-tRNA$_f$ in the absence of Mg^{2+}.

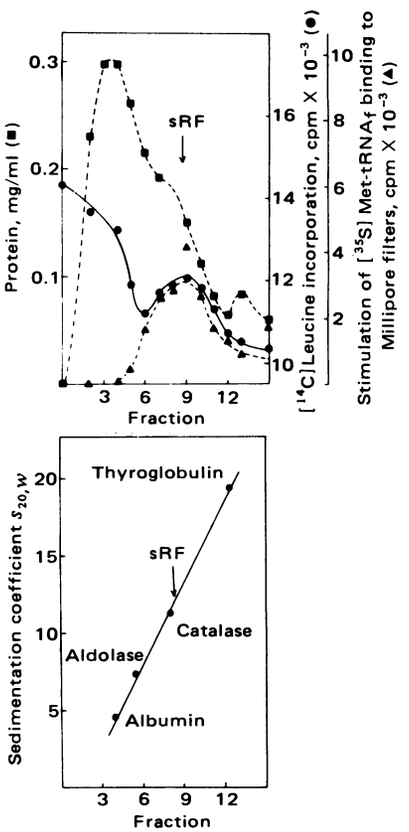

FIG. 3. Glycerol density gradient centrifugation of sRF. sRF (0.9 mg, 100 μl) was centrifuged on a glycerol density gradient as described (8). Protein standards were run in parallel. Amino acid incorporation decreased linearly with glycerol concentration when blank gradients were used. Reversal of ternary complex inhibition (stimulation of [^{35}S]Met-tRNA$_f$ binding in the presence of HRI and ATP) was assayed as described for Table 1, using 10-μl aliquots of gradient fractions. RF activity was determined in 5-μl aliquots added to 20-μl protein synthesis reaction mixtures. Addition of 5 μl of solution containing 30% (vol/vol) glycerol (concentration at the bottom of the gradient) lowered [^{14}C]leucine incorporation to approximately 60%. The specific activity of the [^{14}C]leucine was 85 cpm/pmol. [^{14}C]Leucine incorporation was determined in 20-μl aliquots. The gradients were scanned at 280 nm in an ISCO UA-5 absorbance monitor connected to an ISCO density gradient fractionator. The protein concentrations of the sRF gradient fractions were also determined. Sedimentation coefficients and molecular weights for the standards were taken from ref. 42. The molecular weight of sRF was approximated by plotting M_r of the standards vs. depth of sedimentation.

of the 38,000-dalton subunit of eIF-2. When eIF-2 was phosphorylated by preincubation with HRI, the extent of phosphorylation was not affected by addition of sRF (tracks 12–14). When HRI was preincubated with sRF, its ability to phosphorylate eIF-2 was unaffected (tracks 15–17). These results clearly demonstrate that sRF did not dephosphorylate eIF-2(P) or block phosphorylation of eIF-2 by HRI.

In another experiment, the effects of sRF and Co-eIF-2C on ternary complex formation by purified eIF-2(P) were compared. The results are shown in Table 2. As in the case of eIF-2, ternary complex formation by eIF-2(P) was strongly inhibited by Mg^{2+}. sRF stimulated ternary complex formation by eIF-2(P). As expected, Co-eIF-2C did not stimulate ternary complex formation by eIF-2(P) even at saturating concentrations (1). These results clearly demonstrate that reversal of ternary complex inhibition of sRF was not due to Co-eIF-2C.

Another factor, Co-eIF-2A, stimulates ternary complex formation by both eIF-2 and eIF-2(P). The molecular weight of the purified factor is 25,000. However, a heavy form of this

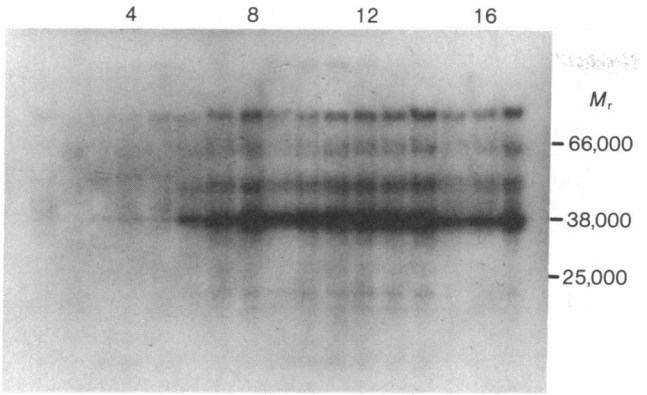

FIG. 4. Phosphorylation of eIF-2 in the presence of sRF. The reaction mixtures (25 μl) contained the same components as the [^{35}S]Met-tRNA$_f$ binding assay mixtures described in Table 1, except that highly purified eIF-2 was used instead of partially purified eIF-2. Incubation was at 37°C for the time indicated. The specific activity of the [γ-^{32}P]ATP used was 2400 cpm/pmol. The reaction mixture was subjected to NaDodSO$_4$/polyacrylamide gel electrophoresis and autoradiography; exposure was for 6 hr. Track 1, HRI (0.4 μg), 10 min; track 2, eIF-2 (0.4 μg), 10 min; track 3, sRF (8 μg), 10 min; track 4, eIF-2 + sRF, 10 min; track 5, HRI + sRF, 10 min; tracks 6–8, HRI + eIF-2, 2, 5, and 10 min, respectively; tracks 9–11, HRI + eIF-2 + sRF, 2, 5, and 10 min, respectively; tracks 12–14, HRI + eIF-2, 10 min, followed by addition of sRF and further incubation for 2, 5, and 10 min, respectively; tracks 15–17, HRI + sRF, 10 min, followed by addition of eIF-2 and further incubation for 2, 5, and 10 min, respectively.

factor (M_r 200,000) has also been observed (6). Co-eIF-2A is stable to heat and *N*-ethylmaleimide (MalNEt). In order to determine whether stimulation by Co-eIF-2A present in sRF was responsible for reversing ternary complex inhibition by HRI, sRF was treated with heat or MalNEt and its ability to reverse ternary complex inhibition was determined. Table 3 clearly shows that the ability of sRF to reverse ternary complex inhibition was abolished by heat or MalNEt. In adddition, the RF activity of sRF was completely abolished by similar heat or MalNEt treatment (data not shown). Reversal of inhibition of ternary complex formation by sRF was not due to Co-eIF-2A.

DISCUSSION

The results presented here clearly demonstrate that the supernatant factor, sRF, that reverses inhibition of protein synthesis in heme-deficient lysates also reverses inhibition of ternary complex formation by HRI. sRF stimulates ternary complex formation under experimental conditions in which eIF-2 is phosphorylated by HRI. sRF also stimulates ternary complex formation by purified eIF-2(P) as well as by the nonphosphorylated form. This stimulation is not due to dephosphorylation of eIF-2(P) by sRF. Also, sRF does not prevent phosphorylation of eIF-2 by HRI. These results suggest that sRF can promote protein synthesis even in the presence of inhibitory concentrations of HRI, with eIF-2(P) as an active initiation factor.

Table 3. Effects of MalNEt addition and heat on sRF activity

Additions	[^{35}S]Met-tRNA$_f$ bound, pmol	
	−HRI −ATP	+HRI +ATP
eIF	1.4	0.7
eIF + sRF	2.0	1.6
eIF + heated sRF	1.5	0.7
eIF + MalNEt-treated sRF	1.6	0.6

Standard Millipore filtration assay conditions described for Table 1 were used. Where indicated, sRF was heated at 55°C for 5 min or incubated at 37°C in the presence of 5 mM MalNEt for 5 min. The excess MalNEt was neutralized by addition of dithiothreitol at a final concentration of 10 mM. The heated and MalNEt-treated sRF preparations were inactive in reversing protein synthesis inhibition in heme-deficient lysates.

The supernatant factor sRF described in this paper appears similar to another factor that we now term rRF. rRF is purified from the ribosomal 0.5 M KCl wash and reverses protein synthesis inhibition in heme-deficient lysates in a manner similar to sRF (8). rRF has been purified from the bulk of eIF-2 activity by using GTP-agarose chromatography, and there are indications that rRF also reverses ternary complex inhibition by HRI. Like sRF, rRF sediments at 12–13 S upon glycerol density gradient centrifugation.

These observations raise important questions regarding the basic mechanism of peptide chain initiation and the regulation of protein synthesis in eukaryotic cells. Despite numerous reports from several laboratories, the detailed mechanism of peptide chain initiation and characteristics of the protein factors involved are not clearly understood. The first step in peptide chain initiation is the formation of a ternary complex, Met-tRNA$_f$·eIF-2·GTP. Reports from our laboratory (4–7) and elsewhere (27–29) indicate that ternary complex formation by eIF-2 is regulated by several protein factors, although the precise roles of these factors in protein synthesis initiation are not known. Formation of the ternary complex by eIF-2 is stongly affected by Mg^{2+}. Ternary complex formation by homogeneous preparations of eIF-2 is optimal in the absence of Mg^{2+} and is strongly inhibited by Mg^{2+}. However, once the ternary complex is formed, the complex is stable to further addition of Mg^{2+}. Co-eIF-2C relieves Mg^{2+} inhibition of ternary complex formation, producing an apparent effect of stimulation (7). This factor would, therefore, appear to be required for protein synthesis initiation at physiological Mg^{2+} concentrations.

HRI phosphorylates the 38,000-dalton subunit of eIF-2. Ternary complex formation by eIF-2(P) is not stimulated by Co-eIF-2C (1, 7, 27–29). It has been suggested that loss of interaction of eIF-2(P) with Co-eIF-2C is responsible for the inhibition of ternary complex formation by HRI.

However, the precise roles of different factors in the formation of initiation complexes with 40S ribosomal subunits are not known. A high molecular weight factor, Co-eIF-2B, promotes dissociation of the ternary complex in the presence of high Mg^{2+} concentration (5 mM) and low temperature (0°C). In the presence of eIF-2, Co-eIF-2B preparations give 3-fold stimulation of Met-tRNA$_f$ binding to 40S ribosomes (4). Presumably, some component in this high molecular weight protein complex [Co-eIF-2B is composed of multiple (16–20) polypeptides and has molecular weight of approximately 450,000] is necessary for 40S initiation complex formation. However, the relationship of this component to the factor that promotes dissociation of the ternary complex is not clear. The ternary complex formed with eIF-2(P) is not dissociated by Co-eIF-2B and is also inactive in 40S initiation complex formation. This observation suggests that the ternary complex dissociation activity in Co-eIF-2B may be involved in 40S initiation complex formation, possibly releasing eIF-2 after Met-tRNA$_f$ is bound to the 40S ribosomal subunit.

The role of Co-eIF-2C in 40S initiation complex formation is not clear. Co-eIF-2C presumably restores the active conformation of eIF-2 in the presence of Mg^{2+} and may play an important role in peptide chain initiation. An alternative possibility is that Mg^{2+} alters the conformation of Met-tRNA$_f$ such that an additional factor (Co-eIF-2C) is required for Met-tRNA$_f$ binding to eIF-2. However, it is conceivable that other factors

besides Co-eIF-2C may perform similar functions. Possibly, sRF is such a factor.

sRF reverses protein synthesis inhibition in heme-deficient lysates and also reverses ternary complex inhibition by HRI. sRF does not dephosphorylate eIF-2(P) or protect eIF-2 from phosphorylation by HRI. sRF stimulates ternary complex formation by both purified eIF-2 and eIF-2(P). The characteristics of stimulation of ternary complex formation by sRF are thus clearly different from those observed with Co-eIF-2C. Also, unlike sRF, Co-eIF-2C does not reverse protein synthesis inhibition in heme-deficient lysates. The sRF preparation contains some Co-eIF-2B activity. It is possible that sRF alters the conformation of eIF-2(P) so that it is recognized by Co-eIF-2B and is also active in 40S initiation complex formation.

The results presented in this paper provide another example of complex regulation of protein synthesis initiation in eukaryotic cells. During heme deficiency, protein synthesis in reticulocyte lysate is inhibited due to activation of HRI. However, a careful analysis of the data will reveal that a low but detectable rate of protein synthesis continues over a prolonged period in these heme-deficient lysates. It is conceivable that sRF activity in these lysates is responsible for the continued low rate of protein synthesis in the presence of inhibitory concentrations of HRI. The presence of eIF-2(P) phosphatase activity in reticulocyte lysate has also been reported (43) and may provide yet another mechanism of reversal of protein synthesis inhibition in heme-deficient lysates. However, sRF does not contain any eIF-2(P) phosphatase activity, and, also, sRF stimulates ternary complex formation even when eIF-2 is phosphorylated. These observations suggest that the sensitivity of protein synthesis to phosphorylation of eIF-2 by HRI may be modulated by the concentration and activity of sRF.

In conclusion, we propose a dual mechanism for regulation of protein synthesis initiation in eukaryotic cells. Both Co-eIF-2C and sRF promote ternary complex formation by eIF-2 at physiological Mg^{2+} concentrations. We suggest that, in rapidly growing cells, Co-eIF-2C may represent the dominant factor. In such cases, protein synthesis would be expected to be highly sensitive to eIF-2 kinases, as is observed in reticulocyte lysates. In resting cells in which eIF-2 kinases may be active, we suggest that the presence of sRF may ensure a continued low rate of protein synthesis necessary for cell survival.

Note Added in Proof. After we submitted our manuscript for review, we noted that Benne *et al.* [Benne, R., Amsez, H., Goumans, H. & Voorma, H. (1979) *XI International Congress of Biochemistry*, p. 129 (abstr.)] have also observed that a postribosomal supernatant factor, similar to sRF, that reverses protein synthesis inhibition in heme-deficient reticulocytes does not dephosphorylate eIF(P) or prevent HRI phosphorylation of eIF-2.

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